ON THE INTERPRETATION OF RATES OF REGENERATION IN TUBULARIA, AND THE SIGNIFICANCE OF THE INDEPENDENCE OF MASS AND TIME[1]

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In his investigations into the metabolic basis of dominance in Tubularia stems and into the factors involved in hydranth regeneration, Barth (1938a, b) proposed as a measure of regeneration rate L/t, in which L is the length of the regenerating primordium and t the time in hours from the removal of the old hydranth to the appearance of a constriction between the primordium of the new hydranth and the rest of the stem. In some cases Barth also used $\pi r^2 L/t$, where r is the radius of the stem; but with uniform short stems r is virtually constant and L becomes an adequate estimate of the mass or volume of tissue involved. Although admitting that variation in primordium length occurs, Child (1940) criticized Barth's definition on the grounds that since growth or increase in cell number is not involved in the reconstitution, the inclusion of mass or volume in the measurement of rate is of doubtful validity. Child therefore maintained that $1/t$ gives a better indication of the kinetics of the process. Miller (1942), contending that Barth's definition is ". . . based on the implied assumption that length and time are inversely related to one another," also questioned the validity of the "implied assumption" on the basis of experiments in which length varied although time did not. Needless to say, Barth's definition of regeneration rate no more implies an inverse relation between the components of the ratio than the usual definition of velocity implies an inverse relation between distance and time.

It is important to note that $1/t$, commonly used as a measure of rate, is not free of ambiguity. Since the "one" in the numerator is a dimensionless quantity, it is clear that the magnitude defined by $1/t$ is not a rate in the generally accepted sense. Ordinarily other dimensions such as mass or length are involved, either alone or in combination, in determination of rate.[2] This is particularly true when such dimensions enter into the process being studied. Regeneration and differentiation, for example, while not usually accompanied by a net mass increase (growth), do imply the occurrence of transformation of mass from one type into another. Presumably these transformations can ultimately be referred to the formation of certain types of compounds at the expense of others. On this basis, then, a rational definition of rate of development would consider the mass of tissue transformed, and might be formulated in terms of mass per unit time. It is admitted that in many cases the difficulty of obtaining mass or volume measure-

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[2] The familiar exception is angular velocity, which has the form $1/t$. Even here however the "one" in the numerator is a consequence of the definition of an angle, which is the ratio of two lengths and therefore dimensionless.

ments of the differentiated tissue has made the use of the $1/t$ definition of rate necessary; nevertheless it may be of value to recognize that $1/t$ does differ from other rates, and to attempt a more precise interpretation of its significance. An analysis of this kind becomes of paramount importance in those investigations which seek to correlate metabolic and developmental rates. Such physiological rates as Q_{O_2}, Q_{CO_2}, or $Q^{N}_{O_2}$ involve a measurement of mass as well as time. Uncritical comparisons between these rates and $1/t$, which neglect the mass transformed, may well lead to erroneous conclusions.

In a previous study of the effects of various respiratory inhibitors on regeneration and respiration rates, the present authors (Moog and Spiegelman, 1942) used Barth's definition of regeneration rate in establishing that with certain inhibitors (e.g., urethanes, azide) rather drastic decreases in regeneration rates can occur without any concomitant measureable effects on Q_{O_2}. But subsequent experiments by Moog (1942) and Spiegelman (1942) indicated clearly that length and time may be independently affected, and so brought out the inadequacy of L/t expression of regeneration rate as a means of comparing directly the results obtained with a variety of agents. A similar situation was noted by Miller (1942), and also by Spiegelman and Goldin (1944) in interpreting the parallel effects of pH variation on regeneration and respiration rates.

Thus it seemed desirable to re-examine the extent of the independence of L and t under different experimental conditions in a more systematic way than had previously been attempted. On the basis of the data obtained and presented here, the significance of L/t as a rate measure will be examined, and the independent variation of L and t will be interpreted in terms of synthetic reactions in open systems approaching the steady state.

Materials and Methods

The solutions used were made up fresh each week in filtered sea water, and when necessary were adjusted to pH 8.2 with hydrochloric acid. Young unbranched stems uniform in translucence, length, and diameter were selected from colonies freshly gathered from the waters of Vineyard Sound or Cape Cod Bay during the months of July and August. Stem segments 6 mm. in length were cut from regions about five mm. proximal to the hydranth. Groups of 25 stem segments were kept in 100 ml. of the appropriate solution in partly filled, tightly stoppered flasks which were shaken at intervals to redistribute the oxygen. Solutions were changed daily, but the stems were kept in the flasks until they reconstituted or were finally transferred to fresh sea water, after four or five days. They were counted as totally inhibited, with rate of regeneration zero, if after being transferred they developed hydranths. In the temperature experiments, the desired temperatures, held constant to 0.5° or better, were obtained with water baths or incubators placed in cold rooms. Solutions in which stem segments were placed were brought to temperature before use.

Results

A. Narcotics

Table I summarizes the results obtained with different narcotics at various concentrations. In the case of ethyl urethane decreases are not observed in re-

TABLE I

The effects of narcotics on regeneration of Tubularia

Concentration (moles/liter)	Number of stems	Time (hours)	Rate		Length		Length/time	
			$1/t$	% of control	Micra	% of control	L/t	% of control
Ethyl urethane								
1. Control	23	38.1	0.0262	100.0	908	100.0	23.8	100.0
1×10^{-3}	24	37.0	0.0271	104.0	958	104.0	25.9	108.0
5×10^{-3}	23	38.8	0.0257	98.0	957	104.0	24.7	104.0
8×10^{-3}	19	39.6	0.0253	96.5	957	104.0	24.2	102.0
1×10^{-2}	22	49.7	0.0201	76.7	928	102.0	18.6	77.8
2×10^{-2}	22	56.9	0.0170	65.0	989	109.0	17.4	72.8
8×10^{-2}	25	60.7	0.0165	64.0	1019	112.0	16.8	70.0
2. Control	17	39.8	0.0251	100.0	963	100.0	24.2	100.0
5×10^{-3}	15	38.8	0.0257	102.0	1009	105.0	26.0	107.0
1×10^{-2}	18	38.6	0.0259	103.0	989	103.0	25.6	103.0
2×10^{-2}	17	52.8	0.0189	75.2	922	95.6	17.5	72.0
3×10^{-2}	11	59.3	0.0168	67.0	1016	106.0	17.1	70.5
4×10^{-2}	10	71.5	0.0140	55.7	981	102.0	13.8	57.0
3. Control	17	43.1	0.0232	100.0	949	100.0	22.0	100.0
1×10^{-7}	18	48.4	0.0207	89.0	1038	110.0	21.4	97.3
1×10^{-6}	18	44.8	0.0225	96.9	972	102.0	21.6	98.0
1×10^{-5}	17	45.4	0.0221	95.1	986	103.0	21.7	98.9
1×10^{-4}	19	49.5	0.0202	87.0	974	102.0	19.7	89.9
1×10^{-3}	18	44.5	0.0226	97.3	1045	110.0	23.6	108.0
1×10^{-2}	14	49.1	0.0204	87.8	888	93.5	18.1	82.1
5×10^{-2}	9	75.0	0.0137	59.0	764	80.5	10.2	46.5
Phenyl urethane								
4. Control	17	59.6	0.0168	100.0	812	100.0	13.8	100.0
1×10^{-4}	25	46.7	0.0214	127.0	773	94.1	16.5	120.0
1×10^{-3}	20	48.9	0.0205	122.0	914	111.0	18.7	133.0
1×10^{-2}	16	57.7	0.0173	103.0	884	107.0	15.3	111.0
2×10^{-2}	20	63.4	0.0158	94.1	858	105.0	13.5	98.0
3×10^{-2}	15	67.6	0.0148	88.0	767	93.5	11.3	82.0
4×10^{-2}	16	68.3	0.0147	87.5	800	97.5	11.7	84.9
5×10^{-2}	16	68.6	0.0146	87.0	757	92.1	11.0	79.8
5. Control	20	34.8	0.0288	100.0	1009	100.0	28.9	100.0
1×10^{-3}	17	30.3	0.0333	116.0	1100	109.0	36.3	122.0
1×10^{-2}	18	33.8	0.0296	103.0	963	95.4	28.2	98.0
2.5×10^{-2}	21	37.7	0.0266	92.4	822	81.5	21.8	75.0
4×10^{-2}	15	51.4	0.0195	67.6	851	84.2	16.6	57.3
5×10^{-2}	14	53.8	0.0186	64.6	536	53.1	10.0	34.5
6.5×10^{-2}	14	66.2	0.0151	52.4	380	37.6	5.7	19.7
Chloretone								
6. Control	24	26.6	0.0376	100.0	1150	100.0	43.2	100.0
1×10^{-2}	14	53.5	0.0187	49.6	920	80.0	17.2	39.9
1.25×10^{-2}	17	43.0	0.0233	61.9	1002	88.0	23.1	53.4
1.50×10^{-2}	18	46.0	0.0217	57.6	1006	88.0	21.7	50.2
1.75×10^{-2}	24	48.3	0.0207	55.0	878	76.3	18.1	41.9
2×10^{-2}	11	45.7	0.0219	58.1	935	81.3	20.4	47.1
2.25×10^{-2}	6	112.0	0.0089	23.6	560	48.7	5.0	11.6
2.50×10^{-2}	11	100.0	0.0100	26.5	590	51.2	5.9	13.7

generation rate (L/t) until the concentration reaches about 0.1 molar. Although the sensitivity does vary from group to group it is evident that the major effect is on the time to constriction. This is made strikingly apparent by figure 1, which is a plot of both primordium length and time to constriction, expressed as per cent of control, against the logarithm of the molar concentration multiplied by 10^6. Here over a concentration range which produces a 44 per cent decrease in the $1/t$ factor, the lengths of the regenerating primordia remain unaffected.

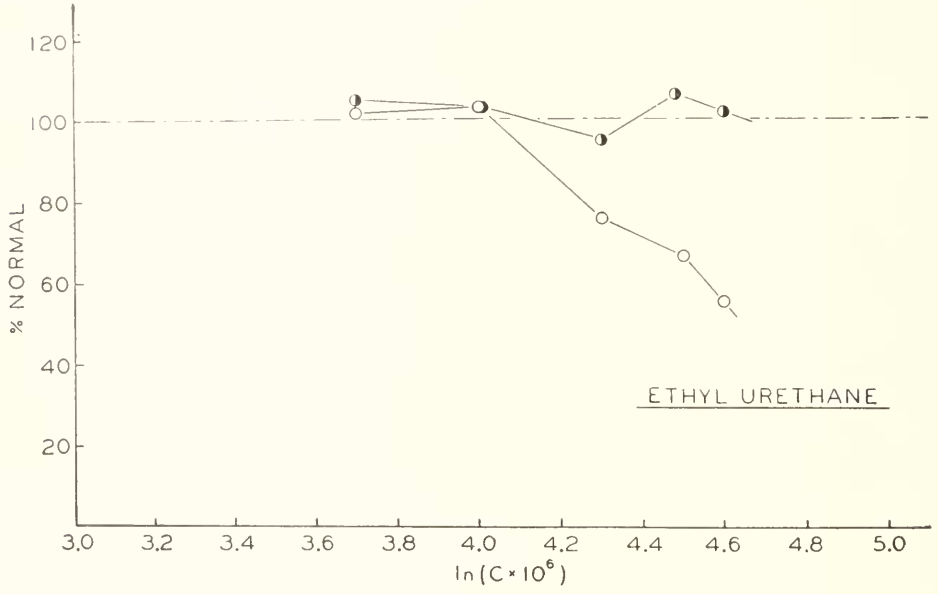

FIGURE 1. The effect of ethyl urethane on rate ($1/t$) of regeneration (open circles) and on length of the regenerating primordium (half-closed circles). Data from experiment 2, Table I.

With both phenyl urethane and chloretone the results are quite different. It is evident from Table I that within the concentration range in which decreases in regeneration rate are obtained, the inhibition involves comparable decreases in both the L and $1/t$ factors. The difference between the data obtained with these two narcotics and that obtained with ethyl urethane is illustrated by figure 2, which represents the data of experiment 6, with chloretone. In comparison with the effects of ethyl urethane, the parallelism of effects here is clear.

B. Cyanide, azide, and oxygen tension.

Tables II and III summarize the data obtained with these reagents. The regeneration rate L/t is extremely sensitive to even relatively low concentrations of cyanide. Thus 6×10^{-6} molar cyanide caused a 17 per cent reduction in L/t, and 5×10^{-5} molar a 61 per cent reduction. However it will be noted that these reductions were due almost entirely to diminishing $1/t$ values. This is illustrated by figure 3, which is a plot of the data of experiment 8; there it may be seen that

in a concentration range which yielded a 61 per cent decrease in the $1/t$ factor the length was affected only to the extent of six per cent. The use of higher concentrations however led quickly to drastic reductions in the amount of tissue transformed.

In the case of azide in the range from 1×10^{-6} molar to 2×10^{-3} molar, the differential effect on the factors of the rate was not as clear-cut as in the case of cyanide. There was again however a tendency for the length to be less sensitive to lower concentrations than $1/t$. Thus in experiment 10, 7×10^{-4} molar azide decreased $1/t$ by 43 per cent and length by eight per cent. In experiment 12, the same concentration resulted in a 38 per cent decrease in $1/t$ and a 12 per cent decrease in length.

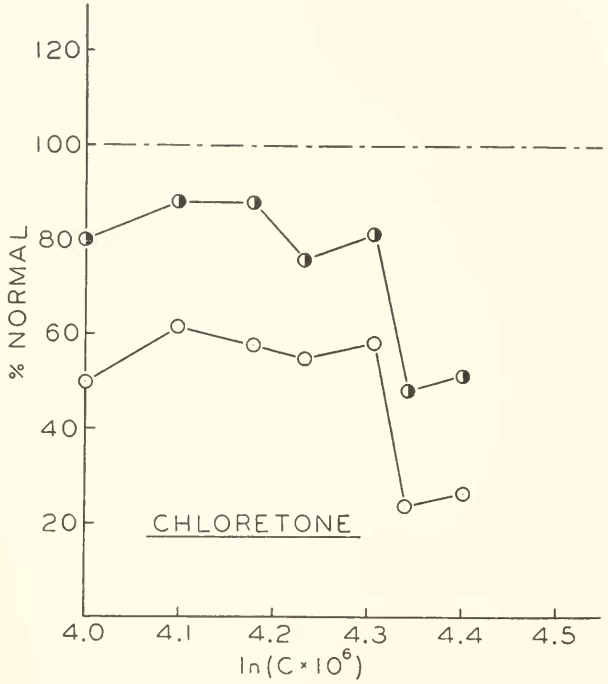

FIGURE 2. The effect of chloretone on rate ($1/t$) of regeneration (open circles), and on length of the regenerating primordium (half-closed circles). Data from experiment 6, Table I.

Both cyanide and azide presumably act by poisoning the cytochrome-cytochrome oxidase system. A study of their effects on both respiration and reconstitution (Moog and Spiegelman, 1942) has however indicated that they follow different pathways in depressing the regeneration process, for cyanide inhibition of reconstitution was always accompanied by a strong depression of the respiratory rate, whereas azide, in a concentration which invariably cuts the reconstitution rate by at least 80 per cent, scarcely altered the rate of oxygen uptake at all. The data presented in Table II further indicate that the ultimate effect of azide differs from that of cyanide. In the case of cyanide a 20 per cent decrease in length is accompanied by a 70 per cent increase in time to constriction, but a

TABLE II

The effects of cyanide and azide on regeneration of Tubularia

Concentration (moles/liter)	Number of stems	Time (hours)	Rate		Length		Length/time	
			$1/t$	% of control	Micra	% of control	L/t	% of control
Sodium cyanide								
7. Control	20	36.4	0.0275	100.0	1175	100.0	32.1	100.0
5×10^{-5}	16	62.9	0.0159	57.7	1025	87.1	16.3	50.7
6.5×10^{-5}	18	76.0	0.0132	48.0	1039	88.2	13.7	42.6
8×10^{-5}	19	88.6	0.0113	40.4	1105	94.0	12.5	39.0
9.5×10^{-5}	18	117.6	0.0085	30.9	953	81.0	8.1	25.2
1.2×10^{-4}	24	128.0	0.0078	28.4	567	48.3	4.4	13.7
8. Control	19	26.2	0.0382	100.0	1176	100.0	44.9	100.0
1×10^{-6}	19	27.1	0.0370	97.0	1193	102.0	44.1	98.3
6×10^{-6}	18	30.8	0.0325	85.1	1146	97.4	37.2	82.3
2×10^{-5}	16	37.2	0.0269	70.5	1170	99.4	31.4	70.0
5×10^{-5}	20	63.9	0.0157	41.1	1103	93.9	17.3	38.5
7.5×10^{-5}	17	123.6	0.0081	21.3	889	76.1	7.3	16.2
9×10^{-5}	13	168.4	0.0060	15.7	445	37.8	2.6	5.8
9.5×10^{-5}	12	172.2	0.0058	15.2	380	32.2	2.1	4.7
1×10^{-4}	20	209.5	0.0048	12.6	141	12.0	0.7	1.6
Sodium azide								
9. Control	19	46.4	0.0215	100.0	986	100.0	21.3	100.0
2×10^{-6}	18	37.4	0.0268	125.0	1003	102.0	26.8	126.0
7×10^{-6}	17	41.6	0.0241	112.0	1013	115.0	24.3	114.0
2×10^{-5}	13	43.7	0.0229	106.0	1013	102.0	23.0	108.0
7×10^{-5}	18	48.5	0.0206	96.1	948	96.0	19.5	91.5
2×10^{-4}	14	71.9	0.0139	65.0	803	81.9	11.2	52.6
7×10^{-4}	15	92.6	0.0109	50.8	710	72.0	7.7	36.2
10. Control	18	29.6	0.0338	100.0	1349	100.0	45.2	100.0
1×10^{-6}	17	34.0	0.0294	75.8	1200	89.0	35.3	78.0
1×10^{-5}	17	49.5	0.0201	51.8	1162	86.4	23.5	51.9
1×10^{-4}	17	31.8	0.0314	81.0	1280	95.0	40.2	88.9
4×10^{-4}	18	36.5	0.0274	70.5	1278	94.6	34.8	76.8
7×10^{-4}	17	45.1	0.0222	57.1	1244	92.3	24.4	53.9
9×10^{-4}	13	51.2	0.0195	50.1	1244	85.0	18.8	41.5
9.5×10^{-4}	18	55.9	0.0179	46.0	1029	76.2	15.3	33.8
1×10^{-3}	11	56.7	0.0177	46.0	1031	76.5	12.1	26.7
1.3×10^{-3}	10	62.1	0.0161	41.5	989	73.4	9.1	20.1
11. Control	19	36.6	0.0274	100.0	1138	100.0	31.8	100.0
5×10^{-5}	18	33.7	0.0298	108.0	1079	94.6	31.5	99.2
2×10^{-4}	18	34.8	0.0288	105.0	994	87.2	28.2	88.7
6×10^{-4}	18	40.6	0.0246	90.0	1035	91.0	25.7	80.9
9×10^{-4}	19	55.6	0.0180	65.5	909	79.7	16.3	51.3
1.5×10^{-3}	13	76.1	0.0132	48.1	669	58.7	11.4	35.8
12. Control	18	30.6	0.0328	100.0	1162	100.0	38.0	100.0
2.5×10^{-4}	17	38.6	0.0259	79.0	1121	96.1	29.8	80.4
4×10^{-4}	19	37.6	0.0266	81.0	1059	90.8	28.1	78.4
5.5×10^{-4}	20	41.4	0.0242	74.0	1042	89.5	25.2	70.4
7×10^{-4}	19	49.4	0.0203	62.1	1024	88.0	20.7	57.8
8.5×10^{-4}	19	44.6	0.0224	68.1	886	76.1	19.9	55.5
1×10^{-3}	17	45.7	0.0219	66.9	897	77.0	19.8	55.3
2×10^{-3}	12	62.9	0.0159	48.5	314	27.0	5.0	13.9

TABLE III

The effect of varying oxygen tensions on regeneration of Tubularia (Data from Barth, 1938b)

Oxygen tension (cc./liter)	Number of stems	Time (hours)	Rate		Length		Length/time	
			1/*t*	% of control	Micra	% of control	*L*/*t*	% of control
13. Control*	20	26.2	0.0382	100	1370	100	52.3	100
2.4	20	36.1	0.0277	72	1072	78	29.9	52
3.2	20	28.1	0.0263	93	1284	94	45.8	83
4.1	20	26.8	0.0374	98	1370	100	51.1	93
4.8	20	26.3	0.0381	100	1365	99	52.0	99
8.2	20	24.5	0.0408	107	1640	120	67.0	128
11.3	20	24.6	0.0407	106	1809	132	73.5	140
14.3	20	24.1	0.0415	106	1840	134	76.5	146
16.5	20	23.7	0.0422	111	1846	135	77.9	148

* Dish open to air.

comparable decrease in length by azide poisoning yields only about a 35 per cent increase in time.

In considering the role the oxygen-utilizing system plays in regeneration, it is of interest to examine, from the point of view of this paper, Barth's (1938b)

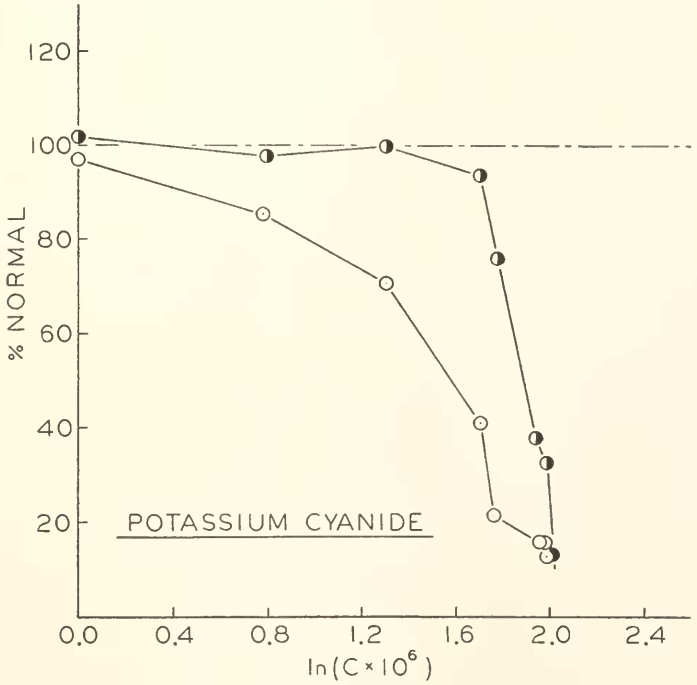

FIGURE 3. The effect of cyanide on rate (1/*t*) of regeneration (open circles), and on length of the regenerating primordium (half-closed circles). Data from experiment 8, Table II.

data on regeneration rates at various oxygen tensions. Table III gives the calculations made from Barth's experiment 7 on young stems comparable to the material used in the present study. There are not enough data on the effect of low oxygen tensions to determine definitely whether unavailability of oxygen acts in the same way as cyanide. The indication is however that cyanide involves other factors, since at 2.4 cc. of oxygen per liter a 22 per cent decrease in length is accompanied by only a 28 per cent increase in time to constriction. The interesting fact to emerge from Table III, in any case, is that at high oxygen

TABLE IV

The influence of temperature on regeneration of Tubularia

Temperature ° C.	Number of stems	Time (hours)	Rate		Length		Length/time	
			$1/t$	% of control	Micra	% of control	L/t	% of control
14. 20.5.....	20	30.8	0.0325	114.0	1100	98.5	35.8	111.9
18.7.....*	*25*	*35.1*	*0.0285*	*100.0*	*1120*	*100.0*	*32.0*	*100.0*
13.5.....	21	47.7	0.0210	73.8	1280	114.0	26.9	84.0
10.8.....	19	57.2	0.0175	61.4	1400	125.0	24.5	76.7
7.0.....	19	95.2	0.0105	36.8	1460	130.0	15.3	48.8
15. 20.5.....	21	39.4	0.0250	109.0	1020	99.0	25.3	106.0
18.7.....	*24*	*43.4*	*0.0230*	*100.0*	*1031*	*100.0*	*23.8*	*100.0*
13.5.....	23	50.0	0.0200	86.9	1180	115.0	23.6	99.1
10.8.....	17	64.5	0.0155	67.4	1290	125.0	20.0	84.0
7.0.....	21	83.4	0.0120	42.2	1380	134.0	16.6	69.6
16. 20.5.....	22	40.0	0.0250	119.0	1010	86.2	25.5	103.4
18.7.....	*23*	*47.6*	*0.0210*	*100.0*	*1174*	*100.0*	*24.6*	*100.0*
13.5.....	24	57.2	0.0175	83.5	1210	103.0	21.2	86.4
10.8.....	18	66.6	0.0150	71.5	1260	107.2	18.9	77.8
7.0.....	18	100.0	0.0100	47.6	1320	112.3	13.2	53.6
17. 20.5.....	21	37.8	0.0265	111.0	1010	93.5	26.8	115.5
18.7.....	*25*	*45.6*	*0.0238*	*100.0*	*1082*	*100.0*	*23.2*	*100.0*
16.0.....	26	48.8	0.0205	86.1	1120	103.3	23.0	99.0
13.5.....	20	55.5	0.0180	75.6	1180	109.0	21.2	91.5
10.8.....	15	71.5	0.0140	58.9	1380	127.0	19.3	83.3
7.0.....	17	100.0	0.0100	42.0	1410	130.2	14.1	60.8

* 18.7° was chosen as the control temperature since it is closest to the natural optimum of the material.

tensions the increased regeneration rate, which can go as high as 148 per cent of normal, results in major part from increases in the mass of tissue transformed. Thus at 14.3 cc./liter the L factor is 34 per cent above normal, whereas the $1/t$ factor is increased only six per cent. This is in sharp contrast to the effect of cyanide, which over a wide range influences the regeneration rate by changing the $1/t$ factor while leaving the L factor relatively unaffected.

C. Temperature

The most striking exhibition of the independence of the length and time factors emerges from the data on the influence of temperature on regeneration,

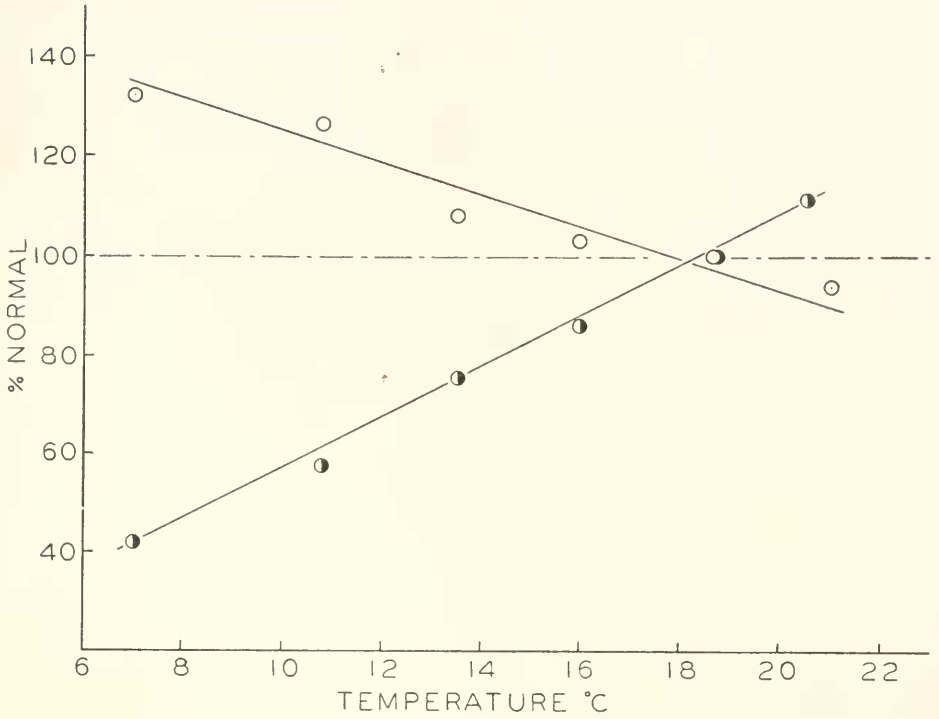

FIGURE 4. The effect of temperature on rate ($1/t$) of regeneration (half-closed circles), and on length of the regenerating primordium (open circles). Data from experiment 17, Table IV.

as summarized in Table IV; the actually opposite effects of temperature on the two rate components are illustrated in figure 4, which is a graph of experiment 17. It is of interest for later discussion to note that, in addition to moving in different directions, the two factors are independently sensitive to temperature changes. Table V shows the Q_{10} values calculated from 7° to 22° C. in five degree intervals. Each value in the "average" column was obtained from the results of four separate determinations. The average value over the entire range is also noted. The high values in each set of four experiments are included in the table to give an estimate of the upper limit of sensitivity for the two factors.

TABLE V

Temperature coefficients (Q_{10}) for length and rate in the regeneration of Tubularia

Temperature range °C.	Length		Rate ($1/t$)	
	Average	High	Average	High
7–12	1.20	1.28	2.14	2.63
12.1–17	1.33	1.37	1.82	1.97
17.1–22	1.16	1.25	1.57	1.76
Average	1.23	1.30	1.88	2.12

It is evident that the Q_{10} values for $1/t$ are consistently higher both in the average and in the highest limits attained than those for L. It will also be noted that Q_{10} for length scarcely changes from low to high temperatures, while on the other hand the coefficient of the $1/t$ factor drops 0.57 from the lowest five degree interval to the highest.

Attempts have been made to explain such differences by invoking two separate processes unlike in their temperature sensitivities, one controlling the mass of tissue transformed and the other the time to constriction. Yet fundamentally length and time may be merely measurements of separate aspects of the same process. From this point of view it is more likely that the true explanation of the different responses to temperature is to be found in the purely numerical character of the terms which determine the two magnitudes, and in the way these magnitudes depend on parameters which vary with temperature. We hope to show in the discussion that this view is quite plausible. For the moment, however, it is sufficient to point out that the independent responses to temperature of L and t serve to emphasize further the independence displayed by the temperature coefficients.

Discussion

That the mass of tissue involved in Tubularia reconstitution is independent of the time to constriction of the new primordium has been demonstrated with a variety of effective agents. It is evident then that L/t cannot be used as a measure of rate under different conditions unless it is accompanied by separate analyses of the behavior of the two components of the ratio. Thus for example the L/t ratio might be found constant over a range of temperature because of inversely proportionate effects on length and $1/t$. It is equally evident that the solution of the problem will not be reached by ignoring one or the other of the factors; for example, $1/t$ would not constitute an adequate description of the effect of high oxygen tension on the regenerative process.

From another point of view L/t, despite its correct dimensionality, may be expected to prove to be a relatively inaccurate measure of transformation rate. For the t in the definition is unlike similar factors in ordinary rate formulae, but is unique in the sense that it is determined by a stage in the development of the system. Its use implies the possibility of measuring rate by taking only two points (zero time and the time to the stage chosen) on the transformation-time curve and using the slope of the line connecting the two points as the rate of the process. Accuracy under such circumstances would be obtained only if the transformation-time curve were perfectly linear, i.e., if the rate up to the stage chosen were constant. In the case of a non-linear curve the approximation would become more and more crude with increasing deviation from linearity as well as with increasing distance between the selected points.

Thus the approximate nature of L/t as a rate measure may be easily recognized; the reasons for the relative independence of the length and time components, however, are not so evident. An insight into some of the factors involved may perhaps be gained by examining an attempt to measure the course of a simple chemical reaction by the same method, i.e., selecting only two points for observation, one at $t = 0$ and the other close to the end of the process, when the system becomes time-independent. So let us assume the following

transformation

$$S \rightleftharpoons H, \tag{1}$$

the forward and backward reactions having velocity constants of k and k' respectively. Let s and h represent the initial concentrations of S and H, and x the number of moles of S transformed into H in t minutes. Then at the end of t minutes, $(s - x)$ is the concentration of S and $(h + x)$ is the concentration of H. After suitable rearrangements the transformation rate at any moment is given by

$$\frac{dx}{dt} = (k_1 - k'h) - (k + k')x. \tag{2}$$

Equation (2) may be integrated to yield the complete time course of the transformation, which takes the form

$$x = A - Ae^{-(k+k')t}, \tag{3}$$

where

$$A = \frac{k_1 s - k'h}{k + k'}. \tag{4}$$

It is evident from equation (3) that as t increases the exponential term becomes smaller and x approaches A, which represents its equilibrium value. Generally in measuring regeneration rates, and particularly in hydranth reconstitution, the stage chosen is one close to the end of the process, beyond which no further significant transformation occurs. A comparable t, in the simplified system being examined, would be one sufficiently large to make the exponential term numerically negligible. Let such a particular t be represented by T. At such time the amount of H present, a measure which would be comparable to the L in the regeneration rate formula, would be given by $(h + A)$. The "rate" analagous to L/t then takes the form

$$\text{rate} = \frac{h + A}{T}. \tag{5}$$

Examination of equations (3) and (4) reveals why the components of a rate so determined may be independent. The magnitude of t which will reduce the value of $Ae^{-(k+k')t}$ sufficiently to make x time-independent obviously depends on the magnitudes of A and $(k + k')$. Any experimental procedure which either increases or decreases k and k' proportionately will leave A, and consequently $(h + A)$, undisturbed, but will change the t (i.e., T) necessary to reduce the second term to insignificance. Under such conditions, the rate as defined by equation (5) would vary solely because of a changing denominator, the mass factor in the numerator remaining constant. On the other hand, an experimental procedure that varied s, the initial concentration of S, might from an observational point of view affect only the mass, since A is a function of s. Strictly speaking, any change in A also influences the T value, since A is included as a factor in the time-determining term. However, in any combination of an ordinary algebraic and an exponential factor, the latter quickly predominates in determining the numerical value of the product. Thus, unless the variation in s produced a very marked change in A, the T values before and after the change

might not be experimentally distinguishable, even though the difference in the A values were easily detected.

It is hardly conceivable that the complex of processes leading to the reconstitution of a hydranth can have much resemblance to the simple reaction represented by equation (1). About the only properties the two have in common are that they both involve molecular transformation and that they both take time to arrive at a time-independent state. The fact that the "rate" of the chemical reaction as defined by equation (5) exhibits many of the characteristics experimentally found for L/t is most likely inherent in the basic similarities underlying the two definitions. The approximate nature of both rate formulations would tend to conceal any differences in the processes they are used to measure. In any case, the above analysis strongly suggests the possibility that some of the peculiarities of the L/t rate found experimentally with the various reagents may in part be characteristic of the definition rather than of the mechanism of regeneration.

It is worthy of note that the decrease in L observed at higher temperatures is not shown by the mass factor of the rate described by equation (5). The numerical reason for the constancy of the numerator with temperature variation is that a temperature change can only yield proportionate changes in the forward and backward velocity constants; since these appear to the same powers in both the numerator and the denominator of A, the net result is that A remains constant. Underlying this behavior is the fundamentally important fact that equation (1) represents a reaction occurring in a closed system and as such its equilibrium point, as far as the concentration of reactants is concerned, is independent of temperature.

If the temperature variation of L is to be examined, therefore, it is necessary to study an open system whose time-independence is maintained by a constant flow of material or energy through it. As has already been pointed out by Burton (1939), open systems are far more likely than closed systems to possess kinetic characteristics typical of living organisms, simply because the latter are themselves open systems, and approach steady states rather than true thermodynamic equilibria. If instead of equation (1) we introduce a source O for S, and a sink P for H, the system becomes an open one, since the concentrations of S and H now become dependent on parameters external to the transformation, namely the levels of O and P. For purposes of simplicity we shall assume that the back reaction k' is either zero or at least negligibly small as compared with the forward reaction. This is plausible whether we consider the S to H transformation itself the energy-yielding reaction which leads to hydranth synthesis, or whether we regard the transformation as being driven by some other energy-yielding reaction. In the first case the transformation would tend to be relatively irreversible, in the second the coupled energy-yielding reaction would tend to make the reverse reaction from H to S relatively insignificant. Instead of (1) then we may write

$$O \overset{k_o}{\rightleftharpoons} S \overset{k}{\longrightarrow} H \overset{k_p}{\rightleftharpoons} P. \tag{6}$$

The velocity constants, k_o and k_p, connecting O to S and H to P respectively, are taken to represent both the forward and the backward velocities of the two

reactions they govern. This assumption of equal forward and backward velocities, while not necessary for the analysis, avoids the undue complication that would result from too many constants. In addition, if O represents a type of source in which S-substrate diffuses from O to the site of the reaction, and P represents a type of sink toward which the produced H diffuses, then the assumption of equality would exactly describe the situation. Letting C_o, C_s, C_h, and C_p represent the concentration levels of O, S, H, and P, the following equation may be written for the kinetics of the transformation of S into H

$$\frac{dC_s}{dt} = k_o C_o - C_s(k_o + k). \tag{7}$$

Integrating equation (7) yields the time variation of the concentration of S, which is given by

$$C_s = B + Ge^{-(k_o+k)t}, \tag{8}$$

where

$$B = \frac{k_o C_o}{k_o + k} \tag{9}$$

and

$$G = (C_{s_o} - B), \tag{10}$$

C_{s_o} being the concentration of S at zero time. Equation (8) is formally identical with equation (3). It is readily seen from equation (8) that B represents the time-independent or steady state value of C_s and is analagous to A of the previous case. Setting up for the present system a rate similar to L/t and equation (5), we may write

$$\text{Rate} = \frac{B}{T} \tag{11}$$

in which T has the same significance as in (5).

Thus again, by the same arguments used in the analysis of a reaction going to equilibrium, which need not be repeated here, it becomes evident that differential effects on either mass or T factor in (11) may be obtained. Thus a suitable experimental procedure which affected only C_o would manifest itself by marked changes in B and weak changes in T. Such may well be the explanation for the results of Barth's experiments on the effects of high oxygen tension, in which the length was increased strongly and the T factor very little. The implication that high oxygen tension raises the level of the source for S (i.e., C_o) fits into the hypothesis proposed by Barth (1940) that oxygen is directly involved in the synthesis of a substance S whose transformation yields hydranth.

Again as in the previous case, treatments which affect the velocity constants k_o and k would result in marked variations in T as compared with B. On the basis of this analysis, one would then interpret the results with ethyl urethane, sodium cyanide, and sodium azide in terms of decrease of the values of velocity constants by poisoning of the enzymes involved in the transformation. Phenyl urethane and chloretone on the other hand, in addition to decreasing the velocity constants, also appear to lower the C_o value by interfering with the synthesis of the substance S or its immediate precursors.

Since increases in temperature raise the values of velocity constants, it is evident why lower T values are found at higher temperatures. A possible reason for the decrease of mass transformed with increasing temperatures may be found in an examination of the effects of variations in k_o and k_1 on B, the steady state value of C_s. The change in B for variations in the velocity constants is given by

$$dB = \frac{C_s(kdk_o - k_o dk)}{(k_o + k_1)^2}. \tag{12}$$

We are concerned here with temperature increases; consequently both dk_o and dk will be increments, i.e., positive quantities. All other factors of the right-hand member of (12) being positive, it is clear that dB will be either positive or negative according as $(kdk_o - k_o dk)$ is either positive or negative. Thus the mass factor will decrease with increasing temperature if

$$kdk_o - k_o dk < 0. \tag{13}$$

Inequality (13) can be satisfied in several ways. Thus for example, if k_o were of the nature of a diffusion coefficient and k the velocity of a chemical reaction, then for a given rise in temperature the increment in k_o would be about half that realized by k. Inequality (13) then becomes

$$dk\left(\frac{k}{2} - k_o\right) < 0$$

and is satisfied if $k < 2k_o$. If on the other hand the two processes have the same temperature coefficients, so that dk were equal to dk_o, inequality (13) could be satisfied if k_o were greater than k. Whatever the intimate details of the situation may actually be, it is evident that opposing responses of mass and time found in regeneration may plausibly be explained as an expression of the operation of an open system approaching a steady state. Decreases of size with increasing temperature are not confined to regeneration in Tubularia, but are a general phenomenon of development and have been studied in other forms including the trout (Gray, 1928; Merriman, 1935), the whitefish (Price, 1940), and the frog (Chambers, 1908).

The different sensitivities to temperature of length and time apparent in the Q_{10} values given in Table V find their most plausible explanation in the algebraic composition of the two terms that determine them. We have already noted that in the special case in which the Q_{10} values of k_o and k are equal, the Q_{10} for the mass would be unity since proportional increases in these constants would cancel out and leave B unchanged for any temperature rise. On the other hand T, since it is determined by the sum of k_o and k, would be affected more or less strongly according to the magnitude of the temperature change. This same difference in response will be carried over to the more general case where the temperature coefficients of k_o and k differ. By the very nature of the dependence of B on these constants, increases or decreases in the constants cannot result in as marked changes in B as they would in T, which is exponentially dependent on their sum.

It is apparent from this discussion that neither $1/t$ nor L/t can be treated as ordinary rates. However the L/t definition of regeneration rate, if supplemented by a further analysis of the separate behavior of L and $1/t$, can yield interpretable information on the regeneration process. The omission of the mass factor is surely not justifiable on the grounds of "correcting" the L/t definition. The latter will in many cases yield information which the "corrected" rate would miss entirely.

Summary

1. Data are presented which show that various agents produce differential effects on the length of the regenerating primordium of a Tubularia hydranth and on the time to the constriction of the primordium from the rest of the stem.

2. The significance of this independence of length and time for the L/t formulation of regeneration rate is discussed.

3. The differential effects are interpreted in terms of a reaction approaching a steady state in an open system.

4. The criticisms of the L/t definition and the proposed substitution of $1/t$ are discussed in terms of the above analysis. It is concluded that the L/t definition, if supplemented by a further analysis of the independent behavior of L and $1/t$, provides a useful and informative measurement of regeneration.

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